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Developmental toxicity of N-methyl-2-pyrrolidone in rats following inhalation exposure

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Abstract

The developmental toxicity of inhaled N-methyl-2-pyrrolidone (NMP) was studied in Sprague–Dawley rats. Pregnant rats were exposed whole body to NMP vapours at concentrations of 0, 30, 60 and 120 ppm, 6 h/day, on gestational days (GD) 6 through 20. Maternal body weight gain was significantly decreased at 60 and 120 ppm on GD 6–13 and maternal food consumption was reduced at 120 ppm on GD 13–21. No significant difference in the gestational weight change corrected for the weight of the gravid uterus was observed, whatever NMP concentration. There were no adverse effects on embryo/fetal viability or evidence of teratogenicity at any concentration tested. Fetal toxicity indicated by reduced fetal weight was observed at 120 ppm. Thus, the no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity was 30 and 60 ppm, respectively.

Keywords: Developmental toxicity; Rat; N-methyl-2-pyrrolidone; Inhalation

1. Introduction

N-methyl-2-pyrrolidone (NMP) is an extensively used organic solvent with many applications in the electronics, agrochemical and petrochemical industries. It is also found in paint-stripping products (HSE, 1997; Trochimowicz et al., 2001).

NMP has been evaluated for its developmental toxicity in rats by various routes of administration. Becci et al. (1982) reported an increased number of resorptions, decreased fetal weights and increases in several skeletal defects following cutaneous administration of 750 mg/kg NMP during major organogenesis. Oral administration of 500 mg/kg NMP or greater from days 6–20 of gestation resulted in increased incidences of post-implantation loss and malformed fetuses (Saillenfait et al., 2002). Types of malformations included anasarca, anal atresia and cardiovascular and skeletal defects. In contrast, no evidence of developmental toxicity was found in rats exposed (whole body) to 100 or 360 mg NMP/m^3 as an aerosol for 6 h/day during main organogenesis (Lee et al., 1987). In another inhalation study, NMP vapours were administered 6 h daily, on GD 4–20, at the single concentration of 165 ppm (whole body). A slight decrease in fetal body weight and in the skeleton ossification was observed in the absence of maternal toxicity (Hass et al., 1995). In a behavioural developmental toxicity study, rats were exposed whole body to 150 ppm NMP vapours for 6h/day, on GD 7–20. Neither significant maternal toxicity nor decrease in the viability of the offspring were observed. Lower body weights of pups until weaning, delay in some development milestones in the preweaning period (i.e. ear unfolding, incisor eruption, eye opening and surface righting reflex), and impairments of higher cognitive functions related to solving difficult tasks were reported (Hass et al., 1994). In a two-generation toxicity study, additional females were exposed whole body to 116 ppm NMP vapours, for 6 h/day, before and throughout gestation, and their offspring were assessed at term for structural alterations. A reduction in fetal weight was noted in the absence of obvious maternal effects (Solomon et al., 1995). Despite inhalation being one of the major routes of exposure to this chemical in the occupational

Abbreviations: GD, gestational day; NMP, N-methyl-2-pyrrolidone; NOAEL, no-observed-adverse-effect level

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and domestic environment, a definitive no-observed-adverse-effect-level (NOAEL) has not been established for the developmental toxicity of NMP vapours.

Thus, the purpose of the present investigation was to provide further information on the dose–response relationships and the NOAEL for NMP developmental toxicity when administered by inhalation to rats during the embryonic and fetal periods.

2. Materials and methods

2.1. Animals

After 2 weeks of acclimatization, nulliparous female (180–200 g) Sprague–Dawley rats supplied by IFFA CREDO Breeding Laboratories (Saint-Germain-sur-l'Arbresle, France) were housed overnight with adult males (one male:two or three females) from the same strain and supplier. The day that vaginal smears were found to be sperm-positive was considered day 0 of gestation (GD). Mated females were randomly assigned to treatment groups using a randomization system stratified by body weight on GD 0. Mated females were housed singly in clear polycarbonate cages with stainless-steel wire lids and corn cob granules as bedding in rooms maintained at 21ff 2 ffC, a relative humidity of 50ff 5%, and a 12-h light/dark photocycle. For exposures, the females were transferred to stainless-steel wire-mesh exposure cages, and the cages were moved into the chambers. After each exposure, the animals returned to their original cages and ''home'' rooms. Food pellets (UAR Alimentation Villemoisson, France) and filtered tap water were available ad lib., except during exposures.

2.2. Test chemical and treatment

N-methyl-2-pyrrolidone (CAS no. 872-50-4, 5 99.5% pure) was purchased from Merck (Darmstadt, Germany). Groups of 25–26 bred rats (20–25 pregnant) were exposed whole body to 30, 60 or 120 ppm of NMP, 6 h/day, on days 6 through 20 of gestation. Control animals were exposed concurrently to filtered room air in an adjacent chamber identical to those of the treatment groups.

2.3. Inhalation exposure

Exposures were conducted in 200-1 stainless-steel inhalation chambers with dynamic and adjustable laminar air flow (6–10 m^3/h). In order to prevent any leakage of the test atmospheres, the chambers were maintained at a negative pressure of no more than 3 mm water. The chamber temperature was set at 23ff 0.6 ffC and the relative humidity at 40ff 5.4%. The system of vapors generation consisted in delivering a constant rate of liquid NMP with an infusion pump to the top of a heated (95 ffC) glass column filled with glass beads. Compressed air (10–30 1/min) heated by a glass heater was introduced at the bottom of the glass column in a counter-current fashion to the liquid NMP. Food and water were withheld during exposures.

Concentrations of NMP were monitored continuously with a gas chromatograph (Intersmat IGC 120 FB) equipped with a flame ionization detector and an automatic gas sampling valve. The column temperature was maintained at 180 ffC. In addition, the exposure levels were determined once during each 6-h exposure period by collecting atmosphere samples through glass tubes packed with resin XAD7. The resin samples were then desorbed with solvent (95% acetone, 5% water) and were analysed by gas chromatography using N-vinyl-2-pyrrolidone as internal standard. Samples were chromatographed on a 2-mm i.d.ffl2 m long stainless-steel column packed with Carbowax 20M on 80/100 mesh Chromosorb W/HP. Column and injector temperatures were maintained at 160 and 250 ffC, respectively.

As NMP has a low vapour pressure (0.27 mm Hg at 20 ffC), the presence of liquid particles was evaluated at the highest concentration generated (i.e. 120 ppm). Airborne particles were measured with an optical particle counter (GRIMM 1105, GRIMM LABORTECHNIK, Ainring, Germany), with a lower detection limit of 0.75 mm. No difference in particle counts was observed between the clean filtered air (control) and vapour-laden air in the exposure chamber. Preliminary level-setting studies have indicated that 120–140 ppm NMP was the highest reliable vapour concentration technically possible.

Because the concentrations determined by analyses were essentially the same as the target concentrations, the target concentrations will be referred to throughout this paper. The actual concentrations (ff SD) (from charcoal tubes) were 30 (ff 1), 60 (ff 2) and 121 (ff 4) ppm.

2.4. Maternal and fetal evaluations

Food consumption was measured for the intervals GD 6–13 and 13–21. Maternal body weights were recorded on GD 0, 6, 13 and 21. On GD 21, the females were killed with an intrapulmonary injection of T61 (Hoechst, Frankfurt, Germany). The uterus was then removed and weighed. The number of corpora lutea, implantation sites, resorptions, and dead and live fetuses were recorded. Uteri with no visible implantation sites were stained with ammonium sulfide (10%) to detect very early resorptions (Salewski, 1964). Live fetuses were weighed, sexed, and examined for external anomalies including those of the oral cavity. Half of the live fetuses from each litter was preserved in Bouin's solution and examined for internal soft tissue changes (Wilson,

1965; Barrow and Taylor, 1969). The other half was fixed in ethanol (70%), eviscerated, and then processed for skeletal staining with Alizarin Red S for subsequent skeletal examination (Staples and Schnell, 1964).

2.5. Statistical analysis

Whenever possible, the data were presented as mean ± SD. The number of corpora lutea, implantation sites and live fetuses and various body weights were analyzed by one-way analysis of variance, followed by Dunnett's test if differences were found. The frequency of post-implantation loss, dead fetuses, resorptions and alterations among litters was evaluated by using the Kruskal–Wallis test followed by the Mann–Whitney test where appropriate. Rates of pregnancy and incidences of fetal alterations per dose were analysed by using Fisher's test. Where applicable, least-squares analysis

Table 1
Maternal parameters from Sprague–Dawley rats inhaling NMP on GD 6-20

	Concentration (ppm/6 h/day)			
	0	30	60	120
No. treated	25	25	25	26
No. (%) pregnant at euthanization	24 (96.0)	20 (80.0)	20 (80.0)	25 (96.2)
No. deaths	0	0	0	0
Body weight (g) on Day 0	235 ± 18[a]	235 ± 19	243 ± 20	237 ± 24
Body weight change (g)				
Days 0–6 (pretreatment period)	35 ± 11	33 ± 8	30 ± 9	32 ± 10
Days 6–13	31 ± 7	27 ± 9	25 ± 8*	23 ± 7**
Days 13–21	104 ± 22	95 ± 31	96 ± 32	89 ± 22
Days 6–21 (treatment period)	134 ± 27	122 ± 36	122 ± 36	112 ± 25
Absolute weight gain[b]	32 ± 9	28 ± 10	26 ± 11	26 ± 10
Food consumption (g/day)				
Days 0–6	22 ± 2	22 ± 2	22 ± 2	22 ± 2
Days 6–13	23 ± 2	22 ± 1	22 ± 2	21 ± 2
Days 13–21	26 ± 3	24 ± 2	25 ± 3	24 ± 2 *
Days 6–21	25 ± 2	23 ± 2	23 ± 2	23 ± 2 *

[a] Values are expressed as means ± SD.
[b] Body weight gain during GD 6–21 minus gravid uterine weight.
*,** Significant differences from the control (air), $P < 0.05$ and $P < 0.01$, respectively.

Table 2
Gestational parameters from pregnant Sprague–Dawley rats inhaling NMP on GD 6–20

		Concentration (ppm/6 h/day)			
		0	30	60	120
All litters[a]		24	20	20	25
	No. of corpora lutea per dam	16.0 ± 2.3[b]	15.7 ± 2.1	16.2 ± 2.6	15.2 ± 1.9
	Mean no. of implantation sites per litter	14.3 ± 3.9	13.4 ± 5.0	14.1 ± 4.6	12.9 ± 4.1
	Mean % post-implantation loss per litter[c]	2.7 ± 3.7	5.2 ± 6.2	9.9 ± 22.3	7.0 ± 9.4
	Mean % dead fetuses per litter	0.0 ± 0.0	0.9 ± 4.2	0.0 ± 0.0	0.0 ± 0.0
	Mean % resorption sites per litter	2.7 ± 3.7	4.3 ± 4.1	9.9 ± 22.3	7.0 ± 9.4
Live litters[d]		24	20	19	25
	Mean no. of live fetuses per litter	13.9 ± 3.8	12.6 ± 4.7	14.0 ± 3.4	12.0 ± 4.1
	Mean % male fetuses per litter	46.6 ± 16.2	60.2 ± 17.7	48.7 ± 12.9	48.6 ± 16.9
	Fetal body weight (g)				
	All fetuses	5.67 ± 0.37	5.62 ± 0.36	5.47 ± 0.25	5.39 ± 0.45*
	Male fetuses	5.81 ± 0.39	5.74 ± 0.32	5.64 ± 0.21	5.52 ± 0.44*
	Female fetuses	5.54 ± 0.37	5.42 ± 0.47	5.32 ± 0.30	5.21 ± 0.44*

[a] Includes all animals pregnant at euthanization.
[b] Values are expressed as means ± SD.
[c] Resorptions plus dead fetuses.
[d] Includes all animals with live fetuses at euthanization.
*Significant difference from the control (air), $P < 0.05$.

was carried out. The reported level of statistical significance was $P < 0.05$. The litter was used as the basis for the analysis of fetal variables.

3. Results

All the animals survived the exposure. Inhaled NMP produced concentration-related reductions in maternal body weight gain on GD 6–13 ($P < 0.01$) and 6–21 ($P < 0.05$), and in food intake on GD 6–13, 13–21 and 6–21 ($P < 0.05$). No significant changes in maternal body weight and food consumption were observed at 30 ppm (Table 1). Body weight gain was significantly reduced during the first half of exposure at 60 and 120 ppm. Exposure to 120 ppm also led to a significant decrease in food consumption on GD 13–21. There were no significant differences in the absolute weight gain between the control and the treated groups.

The mean numbers of implantation sites and of live fetuses and the incidences of non-live implants and resorptions were comparable across groups (Table 2). There was a concentration-related decrease in fetal body weights (all, males, and females, $P < 0.01$), which achieved statistical significance at 120 ppm (5–6% lower than control). Malformations occurred in one fetus from control (omphalocele) and in one fetus at 30 ppm (diaphragmatic hernia) (Table 3). Several common external,

Table 3
Incidence of malformations and variations in fetuses of Sprague–Dawley rats inhaling NMP on GD 6–20

Concentration (ppm/6 h/day)	0	30	60	120
Total no. fetuses (litters) examined[a]				
External	334 (24)	252 (20)	266 (19)	301 (25)
Visceral	167 (23)	126 (20)	133 (19)	151 (25)
Skeletal	167 (24)	126 (19)	133 (19)	150 (25)
Malformations				
Omphalocele	1 (1)	0	0	0
Diaphragmatic hernia	0	1 (1)	0	0
External variations				
Tail, angulated	0	1 (1)	0	0
Club foot (bilateral)	2 (2)	2 (2)	2 (2)	2 (2)
No. (%) fetuses with external variations	2 (0.6)	2 (0.8)	2 (0.8)	2 (0.7)
No. (%) litters with external variations	2 (8.3)	2 (10.0)	2 (10.5)	2 (8.0)
Mean % fetuses with external variations per litter	0.6 ff 2.2[b]	0.7 ff 2.1	0.6 ff 1.8	0.5 ff 1.7
Visceral variations				
Dilated renal pelvis	0	1 (1)	0	0
Distended ureter	1 (1)	4 (4)	1 (1)	0
No. (%) fetuses with visceral variations	1 (0.6)	4 (3.2)	1 (0.8)	0
No. (%) litters with visceral variations	1 (4.3)	4 (20.0)	1 (5.3)	0
Mean % fetuses with visceral variations per litter	0.7 ff 3.5	2.6 ff 5.4	0.8 ff 3.3	0
Skeletal variations				
Supraoccipital, incomplete ossification	0	0	0	1 (1)
Hyoid, incomplete ossification or unossified[c]	0	2 (2)	0	0
Sternebrae				
incomplete ossification or unossified	4 (4)	1 (1)	6 (2)	2 (1)
extra ossification site	1 (1)	0	0	0
Rib(s)				
cervical, rudimentary	2 (2)	1 (1)	3 (3)	2 (2)
14th, supernumerary	19 (10)	19 (12)	12 (9)	12 (6)
Cervical arches, incomplete ossification	0	0	0	1 (1)
Thoracic vertebral centra, incomplete ossification	13 (10)	7 (5)	9 (8)	5 (5)
No. (%) fetuses with skeletal variations	37 (22.2)	27 (21.4)	26 (19.5)	20 (13.3)
No. (%) litters with skeletal variations	17 (70.8)	15 (78.9)	16 (84.2)	11 (44.0)
Mean % fetuses with skeletal variations per litter	26.6 ff 25.9	19.8 ff 15.6	18.3 ff 12.0	15.5 ff 24.1
No. (%) fetuses with any variations	40 (12.0)	33 (13.1)	29 (10.9)	22 (7.3)
No. (%) litters with any variations	17 (70.8)	15 (75.0)	17 (89.5)	13 (52.0)
Mean % fetuses with any variations per litter	16.2 ff 20.6	11.2 ff 8.8	10.1 ff 6.4	7.8 ff 9.9

[a] The incidence of individual defect is presented as number of fetuses (number of litters). Only live fetuses were examined.
[b] Mean ff SD.
[c] Unossified = Alizarin Red S negative.

visceral and skeletal variations were observed, with no indication of any adverse effects related to NMP exposure.

4. Discussion

The results of this study indicate that inhalation exposure of rats to NMP vapours during the embryonic and fetal period did not result in selective toxicity to the offspring.

Maternal toxicity was observed at 60 and 120 ppm, as indicated by reductions in body weight gain during the first half of exposure. This effect was transient and no significant decrease was found after deduction of the gravid uterus weight from the overall weight gain during pregnancy. The dams also exhibited a slight reduction in food consumption at the high concentration. Fetal toxicity limited to a decrease in fetal weight occurred at 120 ppm. Thus, the NOAELs for maternal and developmental toxicity are 30 and 60 ppm, respectively.

These findings are in agreement with the fetal growth retardation and the absence of teratogenic effects found in previous studies on the developmental toxic potential of inhaled NMP. In a two-generation study, rats were exposed whole body to 116 ppm NMP, 6 h/day, prior to mating and throughout gestation and lactation (Solomon et al., 1995). Half of the dams were subjected to caesarean section on GD 21 and the remaining litters were evaluated up to weaning. No adverse effects on the viability and morphological development of the offspring were reported, but a decrease in fetal and pup birth weight. Hass et al. (1995) exposed pregnant rats, whole body, to 165 ppm NMP, 6 h per day, from GD 4 to 20. A marginal decrease in fetal weight and a slightly delayed skeleton ossification were noted. In a postnatal study, exposure to 150 ppm NMP on GD 7–20 resulted in a slight reduction in pup body weight from birth to weaning. No significant maternal toxicity or decrease in the offspring viability was observed (Hass et al., 1994).

In contrast to the mild developmental toxic effects observed in the inhalation studies, mortality and structural malformations have been detected in rats following administration of high doses of NMP by dermal application (Becci et al., 1982) and by gavage (Saillenfait et al., 2002). Differences in the rat developmental response to NMP may be ascribed to quantitative and/or qualitative differences in exposure of the embryo/fetus with the route of administration. Studies in humans and rats indicate that NMP is readily absorbed after inhalation, oral and/or dermal exposure, and that it is extensively metabolised prior to excretion in the urine (Midgley et al., 1992; E.I. du Pont de Nemours, 1995; Ursin et al., 1995; Akesson and Jönsson, 1997, 2000; Jönsson and Akesson, 2001; Anundi et al., 2000). It was reported that NMP is transferred to the fetus after inhalation by pregnant rats near term. Levels of NMP were comparable in maternal and fetal blood (Ravn-Jonsen et al., 1992). This information, however, is not available for the dermal and oral routes.

In conclusion, inhalation exposure of pregnant rats to NMP (vapours) during the entire post-implantation phase of gestation is neither teratogenic nor embryolethal. Evidence of developmental toxicity was limited to intrauterine growth retardation that occurred in the presence of maternal toxicity.

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